REMARKS

Prior to entry of this Amendment, Claims 1-6, 21-35 and 51-76 were pending and under consideration. With this Amendment Claims 21, 51-58 and 59 are being amended and Claims 77-84 are being newly added. Accordingly, after entry of this Amendment, Claims 1-6, 21-35 and 51-84 are pending and under consideration. The amendments of the claims and the various rejections outstanding are addressed below, in the order raised in the Office Action.

Amendments of the Claims

Independent Claims 21 and 59 have been amended so that all instances "target sequence" now recite "predetermined target sequence" so as to conform the bodies of these claims with the preambles. Their scope remains unchanged.

The preamble of independent Claim 51 has been amended to delete the expression "hybridization complex" in favor of the expression "substrate cleavable by a FEN-1 polypeptide." Dependent Claims 52-58 have been amended to be consistent with amended Claim 51. As amended, Claims 51-58 more clearly illustrate the features of the molecules being claimed. The scope of these claims remains unchanged.

The amendments are supported throughout the application as originally filed, and therefore do not introduce new matter. For example, at Col. 43, lines 2-5, molecules are described that form, as a result of hybridization, 5'-flap structures that can be cleaved by a FEN-1 polypeptide. Exemplary species of these molecules are referred to throughout the disclosure as "substrates"(see, e.g., Col. 44, lines 1-2 discussing "Flap Substrate 1" and Col. 46, lines 10-27 discussing "Flap Substrate 2").

Claims 77-84 have been newly added. These new claims are directed to methods of detecting the presence of predetermined nucleotide sequences in samples that comprise detecting cleavage of a 3',5'-double flap cleavage substrate. New claims 77-84 find support in the disclosure originally filed at, for example, Col. 11, lines 3-48 and Col. 42, line 64 through Col. 43, line 36, in connection with FIG. 12 and its associated description.

Defective Oath/Declaration

Claims 1-6, 21-35 and 51-73 stand rejected under 35 U.S.C. § 251 because a supplemental oath or declaration under 37 CFR § 1.175(b)(1) has not yet been received by the

Patent Office. Consistent with MPEP § 1444, Applicant will submit a supplemental oath or declaration under 37 CFR § 1.175(b)(1), as requested by the Office, upon an indication of allowable subject matter. Accordingly, Applicant requests that this rejection be held in abeyance until such time as the claims would otherwise be in condition for allowance.

Rejection Under 35 USC § 251

Claims 21-35 and 51-76 stand rejected under 35 USC § 251 as being allegedly based upon new matter added to the patent for which reissue is sought. Specifically, the Office contends that the disclosure as filed lacks support for: (i) a method step for detecting the presence of a "predetermined target nucleic acid" as recited in Claims 21-35 and 74; (ii) a "hybridization complex" as recited in Claims 51-58 and 75; and (iii) a "kit for detecting a target nucleic acid" as recited in Claims 59-73 and 76. Applicant traverses the rejections.

The disclosure as originally filed clearly discloses a method for detecting the presence of a "predetermined target nucleic acid sequence" as recited in Claims 21-35 and 74. For example, at Col. 11, lines 3-48, the original disclosure describes a novel diagnostic assay that comprises contacting a sample believed to potentially contain *a predetermined target polynucleotide sequence* with a probe capable of specifically hybridizing to all or a portion of the target to form a 5'-flap structure which can be cleaved by a FEN-1 polypeptide, incubating the sample with a FEN-1 polypeptide and detecting the release of nucleotides (or polynucleotides) of the 5'-flap stand. As described, the release of the 5'-flap nucleotides or polynucleotides:

> report[s] the formation of a flap structure (or nicked DNA) and thereby report[s] the presence, and optionally quantity, *of the predetermined target polynucleotide sequence* in the sample.

Original disclosure at Col. 11, lines 14-16 (emphasis supplied). Applicant submits these passages adequately describe the methods of Claims 21-35 and 74. To the extent the claims recite a "predetermined *nucleic acid* sequence" and the passages referenced above refer to a "predetermined target *polynucleotide* sequence," Applicant submits skilled artisans would readily recognize that the expression "polynucleotide" provides written description support for the expression "nucleic acid." Accordingly, Claims 21-35 and 74 do not introduce new matter into the instant reissue application.

Regarding "hybridization complex," Applicants do not understand the rejection. Both the specification of the instant reissue application and the incorporated Harrington & Lieber 1995 article[1] describe molecules that act as substrates for FEN-1 polypeptides that comprise three polynucleotides that are hybridized or annealed to one another in a specified geometry. Skilled artisans would immediately recognize that the expression "hybridization complex" as recited in the preambles of Claims 51-58 and 75 is descriptive of these substrates. The "new matter" clause of 35 U.S.C. § 251 *does not* require *ipsis verbis* correspondence between the language of the specification and the language of the claims. See, e.g., *In re Rasmussen,* 211 USPQ 323, 326 notes 5 & 6 (CCPA 1981); *Fujikawa v. Wattanasin*, 39 USPQ2d 1895, 1904 (Fed. Cir. 1991). All that is required is that the disclosure, considered as a whole, reasonably conveys to skilled artisans that the inventor invented the subject matter claimed. This standard has been met. Nonetheless, in the interests of expediting prosecution, Applicant has amended Claims 51-58 and 75 to recite a "substrate cleavable by a FEN-1 polypeptide," rendering the rejection moot.

A "kit for detecting a target nucleic acid" as recited in Claims 59-73 and 76 is also described in the disclosure of the instant reissue application as originally filed. At Col. 43, lines 39-42, the disclosure clearly states that the invention provides kits "for practicing polynucleotide diagnostic assays according to the methods described." As mentioned above, one of the diagnostic methods described is a method for detecting the presence or quantity of a predetermined target polynucleotide sequence in a sample (see, e.g., Col. 11, lines 13-17 and Col. 43, lines 8-12). Accordingly, the kits recited in Claims 59-73 and 76 do not introduce new matter into the instant reissue application.

The Patent Office also alleges that there is no basis in the application for "polynucleotide" cleavage, and that Applicant has failed to address this issue (see, e.g., Office Action at page 6). The Patent Office bases its conclusion on a passage in an article by Harrington & Lieber[2] that teaches that a murine FEN-1 does not cleave a substrate including an RNA 5'-flap.

---

[1] Harrington & Lieber, 1995, "DNA Structural Elements Required for FEN-1 Binding," J. Biol. Chem. 270:4503-4508 ("Harrington & Lieber 1995a").

[2] Harrington & Lieber, 1995, "The Characterization of a Mammalian DNA Structure-Specific Endonuclease," EMBO J. 13:1235-1246 ("Harrington & Lieber 1995b").

Applicant has addressed this issue previously and notes that the claims reflect this observation. Specifically, independent Claim 21 recites that the 5'-polynucleotide probe is *capable of being cleaved by a FEN-1 polypeptide.* Similarly, the first polynucleotide probes of independent Claims 51 and 59 are *capable of being cleaved by a FEN-1 polypeptide.* This language renders moot any concerns the Office may have regarding the ability of FEN-1 polypeptides to cleave flap structures including an RNA 5'-flap strand.

For the reasons discussed above, Applicant submits Claims 21-35 and 51-76 do not introduce new matter into the instant reissue application. New claims 77-84 likewise do not introduce new matter for the same reasons. Accordingly, Applicant request that the rejection of Claims 21-35 and 51-76 under 35 U.S.C. § 251 be withdrawn.

Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 21-25, 31-35, 51-68 and 74-76 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking adequate written description support. The crux of the rejection appears to be grounded in two perceived deficiencies. First, that Claims 21-25, 31-35, 59-68 and 74-76 do not contain limitations that define the structures of the FEN-1 polypeptides, and second, that Claims 21-25, 31-35, 51-68 and 74-76 do not functionally or structurally describe the double flap FEN-1 substrate. Applicant traverses the rejections.

Regarding the structures of the FEN-1 polypeptides, Applicant has previously pointed out that a genus may be adequately described by disclosure of a representative number of species, and that the disclosure as originally filed describes *at least eight species* of enzymes having 5'-flap endonucleolytic activity (FEN-1 polypeptides): Human FEN-1 (SEQ. ID NO: 1), murine FED-1 (SEQ. ID NO: 3), two yeast FEN-1s (SEQ. ID NO: 5[3] and SEQ. ID NO: 7[4]), and nuclear extracts from calf thymus, rabbit reticulocytes, Chinese hamster fibroblasts and Drosophila embryos[5]. The Patent Office contends these species do not adequately describe the genus of

---

[3] Also known as "YKL510".

[4] Also known as "ΔRAD2".

[5] It is clear from the bolded language at page 8 of the Office Action that the Patent Office doubts these nuclear extracts quality as representative species because the "structure" (presumably primary sequences) of the FEN-1 polypetide comprising them are not explicitly disclosed. The Patent Office places too much emphasis on primary sequence. Applicants do not need to disclose, and indeed do not even need to *knows* the primary sequences of their FEN-1 polypeptides. Applicants have disclosed how to obtain the nuclear extracts *and* that

FEN-1 polypeptides because cleavage of a 3',5'-double flap substrate has been demonstrated only with the murine FEN-1 polypeptide. According to the Patent Office, demonstration of cleavage with a single species is insufficient to be representative of the genus of FEN-1 polypeptides. Applicant disagrees.

As an initial matter, it is important to keep in mind that rejected Claims 21-35 and 59-76 are *not* directed to FEN-1 polypeptide molecules *per se*. Rather, Claims 21-35 and 74 are directed to methods of detecting the presence of predetermined nucleic acid sequences utilizing the 5'-flap cleavage activity of FEN-1 polypeptides, and Claims 59-73 and 76 are directed to kits, which are essentially combinations, useful for, among other things, carrying out the claimed methods. The kits comprise as components a FEN-1 polypeptide and polynucleotide probes capable of annealing with a predetermined target sequence to form a substrate including a 5'-flap that can be cleaved by the FEN-1 polypeptide.

Courts have consistently recognized a distinction between the description required to adequately support claims drawn to genuses of molecules *per se* and claims drawn to methods or combinations that utilize genuses of molecules. For example, in *In re Herschler,* the CCPA noted that "claims drawn to the *use* of *known* chemical compounds must have a corresponding written description only so specific as to lead one having ordinary skill in the art to that class of compounds." *In re Herschler*, 200 USPQ 771, 712 (CCPA 1979) (emphasis in original). Significantly, in *Herschler* the court found that the disclosure of a *single species* of corticosteroid was sufficient to provide written description support for a claimed method of enhancing the penetration of steroid compounds generally.

In *In re Fuetterer*, the CCPA expressly noted that the patent laws do not require applicants to discover which species falling within a functionally described genus will function properly in claims drawn to a combination including the genus:

> Appelant's invention is the *combination* claimed and not that certain inorganic salt have colloid suspending properties. *We see nothing in patent law which requires appellant to discover which of all those salts will function*

---

they have the desired 5'-flap endonucleolytic activity. For the subject matter of method claims and kit claims such as instant Claims 21-35, 59-74 and 76, that is all that is required. It is noted that this situation is somewhat analogous to inventions surrounding the discovery of a natural product that defies structural characterization. The fact that its structure is unknown does not prevent its discoverer from claiming it or its method of use.

> *properly in his combination*. The invention description clearly indicates that any inorganic salt which has such properties is useable in his combination.
>
> * * *
>
> We are not persuaded that our conclusion on this point is wrong by decisions of this and other courts relating to the sufficiency of invention disclosure in cases where the applicant is claiming the chemical compounds *per se*.

In re Fuetterer, 138 USPQ 217, 223 (CCPA 1963) (emphasis supplied). The reasons underlying the different description requirements for method of use and/or combination claims and claims drawn to chemical compounds *per se* are aptly articulated in *Fuetterer*:

> If others in the future discover what inorganic salts additional to those enumerated do have such properties, it is clear appellant will have no control over them *per se*, and equally clear his claims should not be so restricted that they can be avoided merely by using some inorganic salts not enumerated by appellant in his disclosure.

In re Fuetterer, 138 USPQ at 223.

Thus, the Patent Office's continued insistence that claims drawn to methods and kits utilizing an art-recognized genus of molecules require the same quantum of descriptive support with respect to the genus of molecules as composition claims drawn to the genus *per se* is at odds with well-settled law. To the extent the Patent Office relies on the *Rochester v. Searle*[6] decision to support its position, that reliance is misplaced. The facts of the *Rochester* decision are inapposite to the instant case. In *Rochester*, the Court held invalid for want of adequate written description claims drawn to a method of using compounds described only by their desired biological function because the disclosure did not describe *any* compounds having the desired function. As acknowledged by the Patent Office, that is clearly not the case here.

The instant situation is similar to *In re Herschler*. Thus, as articulated in *Herschler*, the question germane to the instant rejection is: would the worker of ordinary skill in this art consider the genus of FEN-1 polypeptides to be operative when considering the disclosure of the instant reissue application? See, e.g., *In re Herschler*, 200 USPQ 711, 717 (CCPA 1979) citing *In re Cook*, 169 USPQ 298 (CCPA 1971). The answer is undoubtedly "yes."

---

[6] *University of Rochester v. G.D. Searle & Co.*, 69 USPQ2d 1886 (Fed. Cir. 2004)

At the time the original application was filed, skilled artisans recognized the FEN-1 species disclosed in the instant reissue application as being representative members of a genus of structure specific endonucleases that share structural and functional homology. For example, Harrington & Lieber reported in the literature in 1994 that the amino acid sequence of murine FEN-1 is highly homologous to the RAD2 protein family, and that this structural homology correlates with functional homology:

> In this study we have cloned the murine FEN-1 gene. This is the first characterized DNA structure-specific endonucleases to be cloned from any eukaryote. Interestingly, FEN-1 is highly homologous to the RAD2 protein family. *Within this family, we show that this structural homology can be extended to functional homology as well.*

Harrington & Lieber, 1994, Genes & Development 8:1344-1355 ("Harrington & Lieber 1994") at page 1348, Col. 2. Specific experiments reported in this article demonstrate that two members of the RAD2 family, YKL510 and a truncated form of RAD2 (ΔRAD2), as well as the murine FEN-1, are all structure-specific endonucleases that cleave 5'-flap structures, but not other structures (Harrington & Lieber 1994 at page 1349, Col. 1). The authors further report that other members of the RAD2 family, including *S. pombe* Rad2, *S. pombe* Rad13, and human XP-G are also likely to be structure-specific endonucleases (see *Id.* at page 1349, Col. 1), and that the calf thymus 5'-endonuclease that interacts functionally with DNA Pol ε is likely the bovine analog of murine FEN-1 (see *Id.* at page 1349, Col. 2).

The cleavage activity of these FEN-1 endonucleases is structure-specific, independent of the sequences of the substrates (Harrington & Lieber 1995b at page 1238, Cols. 1-2), independent of flap length (i*d.* at pages 1239-1240) and always occurs in a structure-specific fashion such that only the 5'-flap strand is cleaved (i*d.* at page 1239, Col. 2). Similar structure-specific 5'-flap cleavage activity was demonstrated in nuclear extracts isolated from other species, including rabbit reticulocytes, Chinese hamster fibroblasts and Drosphila embryos (i*d.* at pages 1236-1237). From these studies, the authors concluded that structure-specific FEN-1 endonucleases are evolutionarily conserved (see, e.g.,i*d.* at page 1243, Col. 1).[7]

[7] These specific FEN-1 endonucleases, as well as the homology between their structures and function, are also explicitly taught in the disclosure of the instant reissue application as originally filed (see, e.g., Col. 44, lines 23-27; Col. 46, lines 14-15; and Cols. 46-56).

Thus, at the time the original application was filed, skilled artisans not only recognized the genus of structure-specific endonucleases referred to in the disclosure as FEN-1 polypeptides, but also recognized the disclosed species as being representative members of the genus that share sequence, as well as functional, homology. Despite this disclosure, the Patent Office summarily concludes that written description is wanting. Applicant reminds the Patent Office that *it* bears the burden of giving reasons as to *why* this is the case. *In re Wertheim*, 191 USPQ 90 (CCPA 1976). The Patent Office has provided no reasoning whatsoever as to why it believes skilled artisans, armed with the knowledge in the art and the instant disclosure, would not consider the demonstrated ability of a murine FEN-1 polypeptide to cleave a 5'-flap substrate that includes a 3'-flap representative of the genus of FEN-1 polypeptides. Surely skilled artisans would expect enzyme molecules known to be functionally and structurally evolutionary conserved, and whose function is to cleave specified nucleic structures, to act on the same substrates.

Moreover, as stated *In re Fuetterer*, Applicants are simply not required to ascertain which of all the species uncompassed by a genus will function properly in the claimed methods and kits. *In re Fuetterer*, 138 USPQ at 223. The description clearly indicates what properties are necessary and what genus of compounds possesses those properties. That is sufficient.

It is noted that new Claims 77-84 are free of this rejection. Claims 77-82 recite "means for cleaving a 3',5'-double flap cleavage substrate." Pursuant to Section 112:

> [a]n element in a claim for a combination may be expressed as a means or step for performing a specified function without the recital of structure, material, or acts in support thereof, and such claim shall be construed to cover the corresponding structure, material or acts described in the specification and equivalents thereof.

35 U.S.C. § 112, sixth paragraph. New claim 83 recites FEN-1 polypeptide encoded by a polynucleotide according to any one of Claims 1-3. New Claim 84 recites FEN-1 polypeptides comprising amino acid sequences selected from certain SEQ ID NOs and fragments thereof.

The Patent Office also alleges that Claims 21-35 and 51-76 do no adequately functionally or structurally define the double-flap substrates. Applicants do not understand the rejection. These claims clearly recite which regions of the probes hybridize or anneal to which portions of the target polynucleotide, thereby dictating the three-dimensional structure of the resultant

substrate. It is this three-dimensional structure that is recognized and cleaved by FEN-1 polypeptides, *not* its nucleotide sequence. An exemplary 3',5'-double flap substrate having those features is illustrated in FIG. 12. The nucleotide sequences of the probes necessary to achieve the three-dimensional 3'5,'-double flap structure will be dictated by the nucleotide sequence of the predetermined sequence of interest by rules of complementarity that are well-understood by skilled artisans. Thus, a skilled artisan armed with a specified predetermined target sequence could immediately envisage and design probe sequences capable of hybridizing with the target sequence so as to form a 3',5'-double flap structure cleavable by a FEN-1 polypeptide.

For the reasons discussed above, Applicant submits Claims 21-35 and 51-76 satisfy the written description requirement of 35 USC § 112, first paragraph. Accordingly, Applicant requests that the rejection of Claims 31-35 and 51-76 under 35 USC § 112, first paragraph be withdrawn.

Conclusion

Claims 31-35 and 51-83 are believed to be in condition for allowance. An early indication of the same is therefore kindly solicited.

No fees beyond those submitted in connection with the enclosed Request for Continued Examination (RCE) and Petition for Extension of Time are believed due in connection with this Amendment, however, the Patent Office is authorized to charge any fees that may be required to Deposit Account No. 50-2778 **(Order No. 375461-043USR1)**.

Respectfully submitted,

Date: October 3, 2005

Ann M. Caviani Pease
Reg. No. 42,067

DECHERT LLP
Customer No. 37509
Telephone: 650.813.4800
Facsimile: 650.813.4848

APPENDIX A

1. An isolated polynucleotide encoding a FEN-1 polypeptide as shown in SEQ ID NO:1 or SEQ ID NO:3, or a fragment of said polypeptide having flap endonucleolytic cleavage activity.

2. An isolated polynucleotide, wherein said polynucleotide comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:29-51.

3. An isolated polynucleotide of **claim 2**, wherein said polynucleotide comprises the sequence of SEQ ID NO:28.

4. A host cell comprising the polynucleotide of **claim 1**.

5. A non-mammalian host cell comprising a mammalian FEN-1 polypeptide of **claim 1**.

6. The polynucleotide of **claim 1** that is full length.

21. A method of detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising the steps of:

(a) contacting, under conditions in which a FEN-1 polypeptide exhibits cleavage activity, a sample suspected of containing a target nucleic acid comprising the predetermined target sequence with:

(i) a 5'-polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region that is capable of specifically hybridizing under said cleavage conditions to a first portion of the predetermined target sequence and a 5'-region located immediately 5' to the 3'-region; and

(ii) a 3'-polynucleotide probe comprising a 5'-region that is capable of specifically hybridizing under said cleavage conditions to a second portion of the predetermined target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region,

such that the 3'-region of the 5'-probe and the 5'-region of the 3'-probe specifically hybridize immediately contiguously with one another to the first and second portions,

respectively, of the predetermined target sequence to form a 5',3'-double flap structure cleavable by a FEN-1 polypeptide;

(b) cleaving the 5',3'-double flap structure with a FEN-1 polypeptide; and

(c) detecting the presence or absence of, and/or quantifying the amount of, FEN-1 polypeptide-generated cleavage, thereby detecting the presence of the predetermined target sequence in the sample.

22. The method of **claim 21** in which the 5'-probe contains a detectable label.

23. The method of **claim 22** in which the 5'-region of the 5'-probe contains the detectable label.

24. The method of **claim 23** in which the 5'-end of the 5'-probe contains the detectable label.

25. The method of **claim 21** in which the 5'-probe is immobilized on a support.

26. The method of **claim 21** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

27. The method of **claim 26** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO:28.

28. The method of **claim 21** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

29. The method of **claim 21** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

30. The method of **claim 21** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

31. The method of **claim 21** in which the 3'-region of the 3'-probe is 1 to 10 nucleotides in length.

32. The method of **claim 21** in which the 3'-region of the 3'-probe is 1 nucleotide in length.

33. The method of **claim 21** in which the 5'-region of the 5'-probe is 1 to 5 nucleotides in length.

34. The method of any one of **claim 21-33** in which the amount of FEN-1 polypeptide-generated cleavage is quantified.

35. The method of any one of **claims 21-33** in which the presence or absence of FEN-1 polypeptide-generated cleavage is detected.

51. ~~A hybridation complex~~ A substrate cleavable by a FEN-1 polypeptide comprising:

(a) a bridge polynucleotide comprising a first portion and a second portion located immediately 3' to the first portion;

(b) a first polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region and a 5'-region located immediately 5' to the 3'-region; and

(c) a second polynucleotide probe comprising a 5'-region and a 3'-region located immediately 3' to the 5'-region,

wherein the 3'-region of the first probe and the 5'-region of the second probe are specifically hybridized immediately contiguously with one another to the first and second portions, respectively, of the same bridge polynucleotide molecule, thereby forming a ~~hybridization complex~~ substrate cleavable by a FEN-1 polypeptide.

52. The ~~hybridization complex~~ substrate of **claim 51** in which the first probe contains a detectable label.

53. The ~~hybridization complex~~ substrate of **claim 52** in which the 5'-region of the first probe contains the detectable label.

54. The ~~hybridization complex~~ substrate of **claim 53** in which the 5'-end of the first probe contains the detectable label.

55. The ~~hybridization complex~~ substrate of **claim 51** in which the first probe is immobilized on a substrate.

56. The ~~hybridization complex~~ substrate of **claim 51** in which the 3'-region of the second probe is 1 to 10 nucleotides in length.

57. The ~~hybridization complex~~ substrate of **claim 56** in which the 3'-region of the second probe is 1 nucleotide in length.

58. The ~~hybridization complex~~ substrate of **claim 51** in which the 5'-region of the first probe is 1 to 5 nucleotides in length.

59. A kit for use in detecting the presence of a predetermined target nucleic acid sequence in a sample, comprising:

(a) a FEN-1 polypeptide;

(b) a first polynucleotide probe capable of being cleaved by a FEN-1 polypeptide, comprising a 3'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a first portion of the predetermined target sequence and a 5'-region located immediately 5' to the 3'-region; and

(c) a second polynucleotide probe comprising a 5'-region capable of specifically hybridizing under FEN-1 polypeptide cleavage conditions to a second portion of the predetermined target sequence which is located immediately 3' to the first portion and a 3'-region located immediately 3' to the 5'-region,

wherein the 3'-region of the first probe and the 5'-region of the second probe are capable of specifically hybridizing immediately contiguously with one another to the first and second portions, respectively, of the predetermined target sequence to form a 5',3'-double flap structure that is capable of being cleaved by the FEN-1 polypeptide.

60. The kit of **claim 59** in which the first or second probe contains a detectable label.

61. The kit of **claim 59** in which the FEN-1 polypeptide contains a detectable label.

62. The kit of **claim 59** in which the 3'-region of the second probe is 1 to 10 nucleotides in length.

63. The kit of **claim 59** in which the 3'-region of the second probe is 1 nucleotide in length.

64. The kit of **claim 59** in which the 5'-region of the first probe is 1 to 5 nucleotides in length.

65. The kit of **claim 59** in which the first probe contains a detectable label.

66. The kit of **claim 65** in which the 5'-region of the first probe contains the detectable label.

67. The kit of **claim 66** in which the 5'-end of the first probe contains the detectable label.

68. The kit of **claim 59** in which the first or second probe is immobilized on a substrate.

69. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

70. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide is encoded by a polynucleotide comprising SEQ ID NO. 28.

71. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

72. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

73. The kit of any one of **claims 59-68** in which the FEN-1 polypeptide comprises the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

74. The method **claim 21** in which the 5'-region of the 5'-polynucleotide probe is 20 nucleotides in length.

75. The substrate of **claim 51** in which the 5'-region of the first polynucleotide probe is 20 nucleotides in length.

76. The kit of **claim 59** in which the 5'-region of the first polyncleotide probe is 20 nucleotides in length.

77. A method of detecting the presence of a predetermined nucleotide sequence in a sample, comprising:

contacting a sample suspected of containing a polynucleotide comprising the predetermined nucleotide sequence with (i) oligodeoxyribonucleotide probes capable of forming a 3',5'-double flap cleavage substrate in the presence of the predetermined nucleotide sequence and (ii) means for cleaving the 3',5'-double flap cleavage substrate, wherein the contacting is performed under conditions in which the oligodeoxyribonucleotide probes anneal with the predetermined nucleotide sequence, if present in the sample, to yield the 3',5'-double flap cleavage substrate; and

detecting cleavage of the 3',5'-double flap cleavage substrate, thereby detecting the presence of the predetermined nucleotide sequence in the sample.

78. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide encoded by a polynucleotide comprising a sequence selected from the group of sequences consisting of SEQ ID NOS: 29-51.

79. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide encoded by a polynucleotide comprising SEQ ID NO. 28.

80. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide comprising the amino acid sequence shown in SEQ ID NO:1 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

81. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide comprising the amino acid sequence shown in SEQ ID NO:3 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

82. The method of **claim 77** in which the means for cleaving the 3',5'-double flap substrate is a FEN-1 polypeptide comprising the amino acid sequence shown in SEQ ID NO:5 or SEQ ID NO:7 or a fragment thereof having 5'-flap endonucleolytic cleavage activity.

83. A method of detecting the presence of a predetermined nucleotide sequence in a sample, comprising:

contacting a sample suspected of containing a polynucleotide comprising the predetermined nucleotide sequence with (i) oligodeoxyribonucleotide probes capable of forming a 3',5'-double flap cleavage substrate in the presence of the predetermined nucleotide sequence and (ii) a FEN-polypeptide encoded by a polynucleotide according to any one of **claims 1-3**, wherein the contacting is performed under conditions in which the oligodeoxyribonucleotide probes anneal with the predetermined nucleotide sequence, if present in the sample, to yield the 3',5'-double flap cleavage substrate; and

detecting cleavage of the 3',5'-double flap cleavage substrate, thereby detecting the presence of the predetermined nucleotide sequence in the sample.

84. A method of detecting the presence of a predetermined sequence in a sample, comprising:

contacting a sample suspected of containing a polynucleotide comprising a predetermined sequence with first and second oligodeoxyribonucleotide probes and a FEN-1 polypeptide, wherein: (i) the first oliogodeoxyribonucleotide probe comprises a 5'-flap region and a 3'-region

complementary to a first region of the predetermined sequence; (ii) the second oligodeoxyribonucleotide probe comprises a 3'-flap region and a 5'-region complementary to a second region of the predetermined sequence that is located downstream of, and contiguous to, the first region of the predetermined sequence; (iii) the FEN-1 polypeptide comprises a sequence selected from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and fragments thereof having 5'-flap endonucleolytic cleavage activity; and (iv) the contacting is performed under conditions in which the first and second oligodeoxyribonucleotide probes anneal with the predetermined sequence, if present in the sample, to form a 3',5'-double flap substrate cleavable by the FEN-1 polypeptide; and

detecting cleavage of the 3',5'-double flap substrate, thereby detecting the presence of the predetermined sequence in the sample.